# PROGRESSIVE VACCINIA COMPLICATING LYMPHOSARCOMA

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# PREFACE

The performance of post-mortem examinations is an important part of the work of a practising pathologist. Many of these examinations would appear "straightforward" to the casual observer, but there are few in which a thorough dissection does not reveal some unexpected finding. Occasionally one is confronted by a rarity, and the post-mortem examination takes on greater significance contributing new facts to our understanding of disease processes. This report describes such a case, an example of progressive vaccinia complicating lymphosarcoma; one which stimulated a detailed histological and electron-microscopy study.

Rarity alone, however, is insufficient justification for such a lengthy analysis. The most interesting aspect of this case is the gross immunological "breakdown" whereby the patient succumbed to a fulminant, disseminated infection by vaccinia virus, a virus which the vast majority of subjects localize to the skin and rapidly eliminate. That this breakdown occurred in a patient with lymphosarcoma, and was exaggerated by steroid therapy, has important implications regarding host defences to virus infection. It is these implications that make the case worthy of detailed consideration.

INTRODUCTION

Progressive vaccinia is the rarest and most grave of the complications of anti-smallpox vaccination. In this disorder the normal cessation of virus multiplication by the 10th day after vaccination does not occur and the lesion fails to undergo regression. The vesicle continues to enlarge and forms a sloughing ulcer whose advancing edge is associated with large satellite vesicles. Metastatic lesions appear at distant cutaneous and visceral sites as a result of blood-borne infection, and most cases die. Progressive vaccinia must be distinguished from the more common condition, generalised vaccinia, in which widespread lesions also occur, but these resolve in a normal manner and rapid recovery is the rule.

Most cases of progressive vaccinia have occurred in children and have been associated with low levels or absence of gamma-globulin (Kempe, 1960). In adults, however, a striking association with malignant lymphoreticular neoplasms (reticuloses) and leukaemias is emerging. This association was present in 36 of the 40 adult cases of progressive vaccinia found in the literature.

The present case is an example of progressive vaccinia developing in a patient with lymphosarcoma, an association which has been recorded in only one previous case (Öberg, Nathorst-Windahl and Wesslén, 1958). The case is noteworthy in that it illustrates the exacerbating effects of steroid therapy and previous irradiation on vaccinial infection. In addition this patient /

patient developed a severe vaccinial pneumonia, and the presence of virus in the lungs was confirmed by electron microscopy.

## Clinical Summary

The patient, a man aged 67, first presented in 1963 with a dislocated shoulder. On clinical examination at that time he was found to have enlarged axillary and cervical lymph-nodes and subsequent biopsy of an axillary gland revealed the appearances of lymphosarcoma.

He was treated with radiotherapy on numerous occasions; the last course of irradiation, for enlarged mesenteric glands, being in October 1968. During this period of treatment he developed mild diarrhoea. He was discharged home at the beginning of November 1968.

Shortly after discharge he requested vaccination prior to a trip abroad, and this was carried out by his general practitioner on 15 Nov. 1968. Four days later he appeared for routine follow-up at the Radiotherapy Clinic and haematological investigations indicated the presence of mild haemolytic anaemia. As no one was aware that the patient had been vaccinated, a course of prednisolone, 15 mg per day, was recommended, and the steroid therapy commenced on 27 Nov. 1968 - i.e., 12 days after vaccination.

The patient had previously been vaccinated in infancy and in 1943, and initially developed a mild reaction of immunity. On 1 Dec., however, extension of the inoculation site was noted. Prednisolone was stopped on 3 Dec. and he was put on prophylactic Ampicillin to prevent superinfection.

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The arm lesion became progressively worse and satellite and metastatic vesicles appeared. He again developed diarrhoea and this was attributed to Ampicillin treatment. On 9 Dec. he was admitted to hospital where, despite treatment with hyperimmune gamma-globulin, his condition remained unchanged. Three days later there was a sudden deterioration, and he complained of breathlessness, sweating and vague chest pain with radiological evidence of consolidation in the left basal zone. The clinical impression was of pulmonary embolism possibly secondary to axillary vein thrombosis in the left arm. Despite intensive care he died later the same day, 27 days after vaccination.

There was no evidence during this final illness of progression of the lymphosarcoma to lymphatic leukaemia. The maximum white blood cell count was 9200 per μl with only 15 per cent. lymphocytes.

A blood culture taken on the day of his death produced a growth of Salmonella typhimurium. Mild diarrhoea had been present since his last period of hospitalisation, but this organism had not been isolated from the faeces.

## PATHOLOGICAL CHANGES

### Necropsy Findings

The post-mortem examination was performed 37 hr after death.

Extensive ulceration was present around the vaccination site on the left upper arm. The blackened circular ulcer was 6 cm in diameter and surrounded by a wide zone of blue discoloration. The remainder of the arm and the dorsum of the hand were oedematous, and numerous satellite lesions studded the skin of the arm. /

arm. Scattered metastatic lesions were present, mainly on the trunk and the right arm.

On internal examination, enlarged lymph-glands were found in both axillae, in the mediastinum and throughout the mesentery.

In the cardiovascular system there was no evidence of pulmonary embolism or of myocardial infarction, and dissection of the left subclavian and axillary veins failed to reveal in-situ ante-mortem thrombus.

The lungs were considerably heavier than normal, the left weighing 980 and the right 1040 g. All lobes were markedly affected by pulmonary oedema; both lower lobes felt firm and contained numerous pale areas of consolidation.

The liver (1650 g) and the spleen (350 g) were somewhat enlarged, but showed no macroscopic abnormality.

Vaccinial lesions were not present in the mucosa of the pharynx, oesophagus or trachea.

All other systems were of normal appearance. In particular, the intestines were free of ulceration and there was no apparent enlargement of lymphoid tissue within the bowel wall. The brain was not available for examination.

## Histology

Lymph-glands. The normal architecture of the glands is completely destroyed and is replaced by a solid sheet of lymphocytes. A small proportion of the cells have larger vesicular nuclei and are recognisable as lymphoblasts. Small aggregations of lymphocytes are present outside the capsule within surrounding adipose /

adipose tissue. The appearances are those of lymphosarcoma (fig. 1).

Skin. Sections of skin taken from the edge of the ulcerated area reveal marked thickening of the epidermis with conspicuous vacuolation of epidermal cells and some intra-epidermal vesiculation (fig. 2). The underlying dermis is oedematous and haemorrhagic and the capillaries are dilated and congested. A striking feature is the greatly diminished inflammatory reaction, represented only by a few scattered lymphocytes in the upper dermis (fig. 3).

Examination of sections stained with phloxine-tartrazine, Mann's and Giemsa stains do not reveal unequivocal inclusion bodies, although moderate numbers of cells contain granular eosinophilic collections that are presumably cytoplasmic degenerative material.

Lung. Sections of lung taken from the upper lobes show widespread involvement by proteinaceous oedema with swelling of alveolar walls (fig. 4). The alveoli contain histiocytes presumably shed from the walls. The consolidated areas in the lower lobes present a more unusual appearance. The alveoli are largely obliterated by a dense fibrinous oedema, which again is infiltrated by small numbers of macrophages (fig. 5). Within this consolidated tissue, large necrotic foci containing scattered pyknotic and fragmented nuclei are present (figs. 6 and 7). These areas of consolidation are associated with only a few scattered polymorphs, although small aggregations of lymphocytes are present in association with the bronchi.

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The bronchial epithelium shows marked hyperplasia, being increased in thickness and displaying obvious mitotic activity (figs. 8 and 9). Inclusion bodies are not seen.

Gram and periodic acid-Schiff stains fail to reveal bacteria or yeasts in association with the lung lesions.

Liver. The liver contains numerous scattered foci of necrosis, which consist in the main of a few cells showing cytoplasmic vacuolation and loss of nuclei, but occasional larger circumscribed areas of necrosis are present (fig. 10). Increased numbers of Kupffer cells, many with prominent cytoplasm, are seen. The portal tracts show heavy infiltration by lymphocytes, and occasional lymphocytes are present within sinusoids. The areas of necrosis are not, however, associated with a leucocyte reaction; they are surrounded by a zone of congestion and, in some instances, increased numbers of macrophages. Polymorphonuclear leucocytes and macrophage granulomata are not seen.

Spleen. The spleen is hypocellular and shows complete loss of the normal architecture. Germinal centres are absent and the tissue consists largely of markedly congested red pulp. In some vessels, subendothelial collections of lymphocytes are seen (fig. 11). Polymorphonuclear leucocytes are absent.

Sections of myocardium, thyroid, adrenals and kidneys showed no notable abnormality. Fibrin stains were performed on sections of liver and kidney, but these proved negative.

## Electron Microscopy

Electron microscope examination was carried out on specimens of /

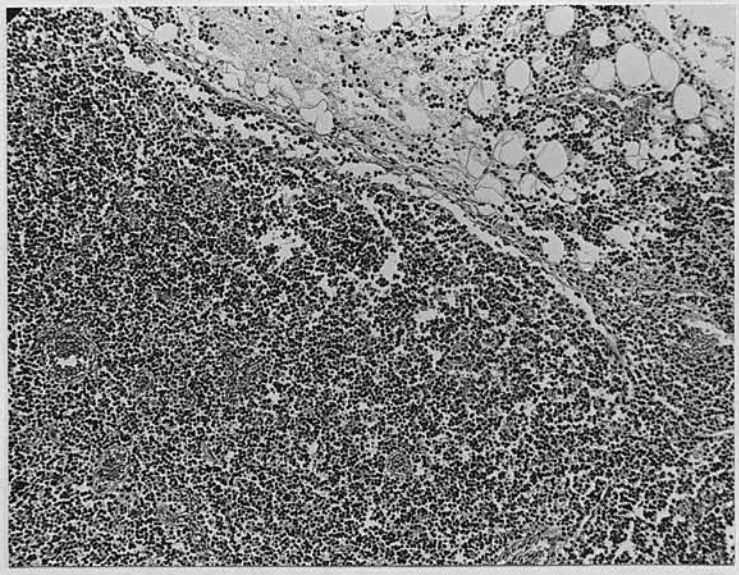

Fig. 1. Lymph-gland. Loss of normal architecture and replacement by a sheet of lymphocytes, with local infiltration into the surrounding adipose tissue. Haematoxylin and eosin. x 75.

Fig. 2. Skin from edge of the ulcer. Epidermis thickened, with "ballooning" of epithelial cells and intra-epidermal vesiculation. HE. x 75.

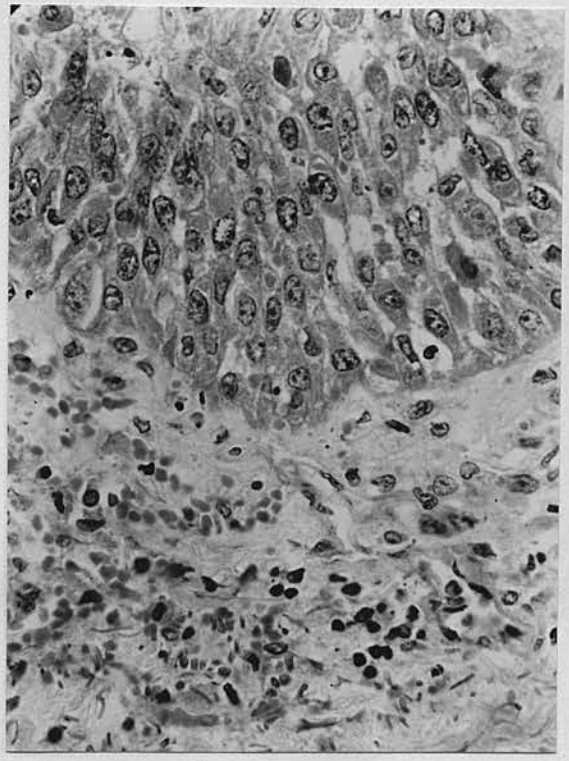

Fig. 3. Skin; epidermal-dermal junction. Edge of ulcer shows in the upper dermis markedly diminished inflammatory reaction with small numbers of lymphocytes. HE. x 320.

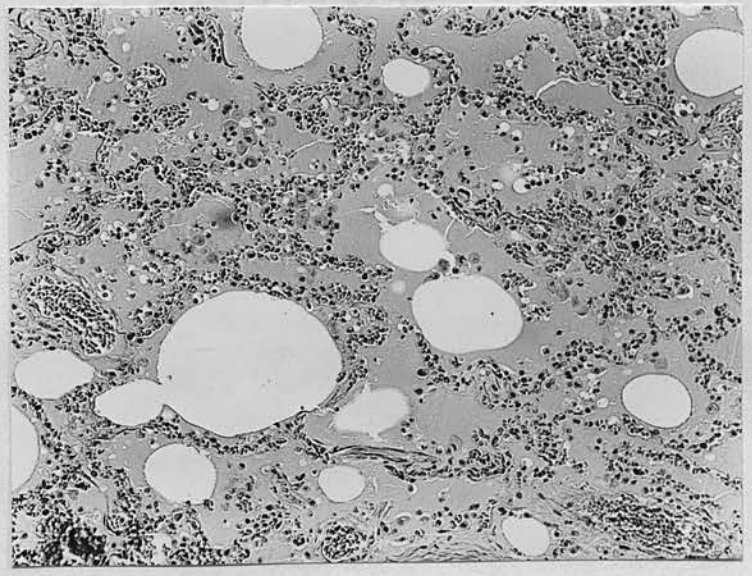

Fig. 4. Lung, right upper lobe. Alveoli filled with proteinaceous exudate containing macrophages. HE. x 95.

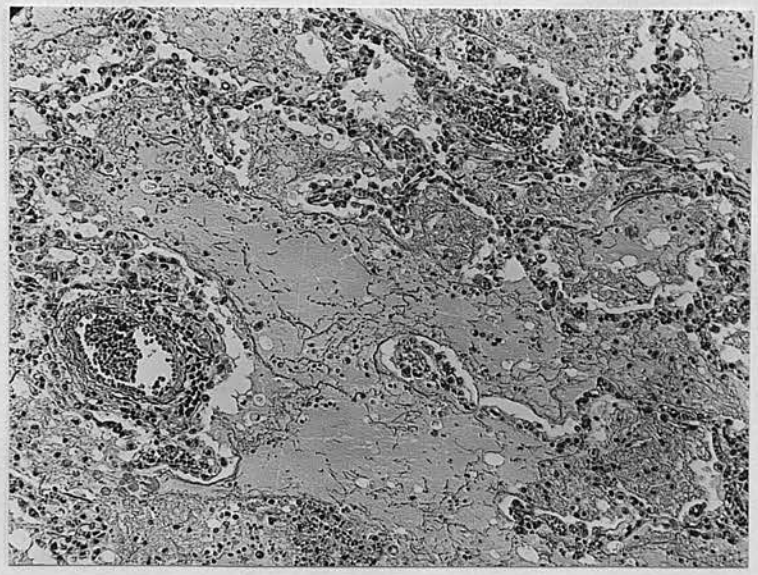

Fig. 5. Lung, lower lobe. Both lower lobes show widespread sero-fibrinous oedema infiltrated by scanty macrophages and lymphocytes. HE. x 95.

Fig. 6. Lung, lower lobe. Necrotic focus containing conspicuous strands of fibrin, macrophages and numerous pyknotic and fragmented nuclei. HE. x 95.

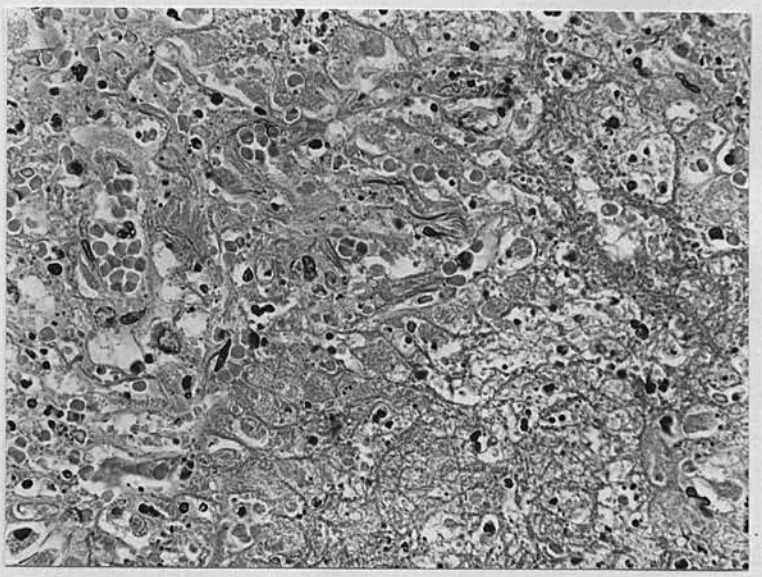

Fig. 7. Lung. Necrotic area with coarse meshwork of fibrin, granular degenerate cells devoid of nuclei, macrophages, and nuclear debris. HE. x 320.

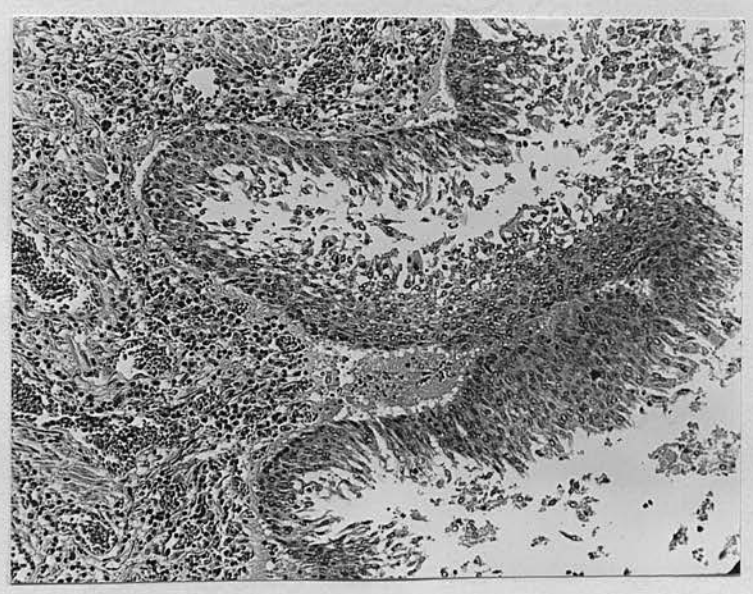

Fig. 8. Hyperplastic epithelium within a medium-sized bronchus. HE. x 95.

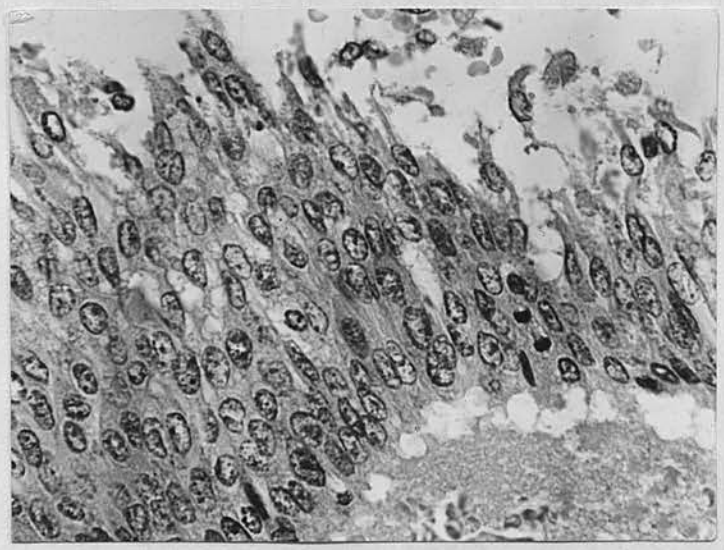

Fig. 9. Bronchial epithelium. Stratification of epithelium and a mitotic figure. HE. x 425.

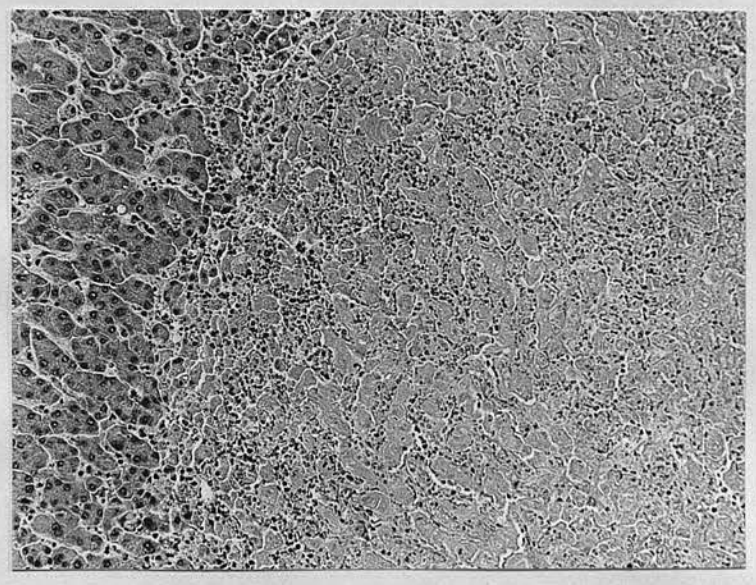

Fig.10. Part of a large focus of necrosis in the liver. HE. x 95.

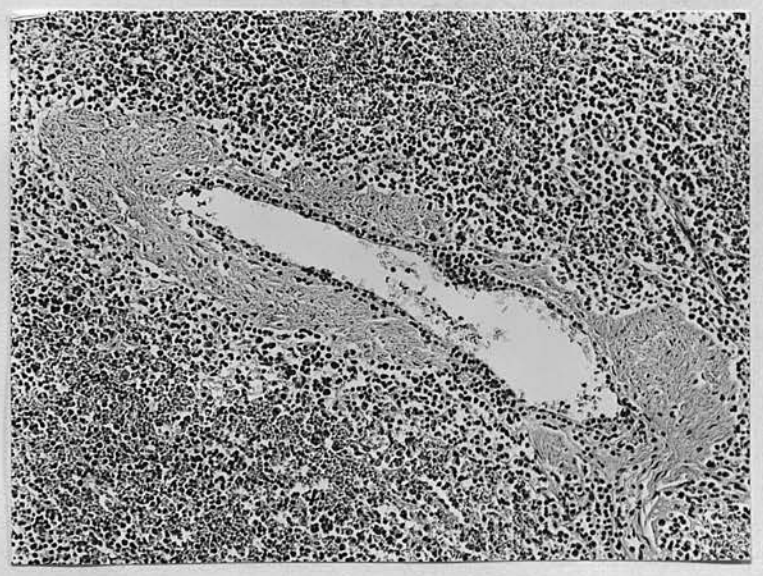

Fig.11. Small splenic vessel with dense surrounding cuff and sub-endothelial collections of lymphocytes. HE. x 95

of skin and lung.

The tissue had initially been fixed in corrosive formol solution for routine histological examination and had therefore to be specially processed prior to electron microscopy.

The tissues were cut into small pieces each about 1 cu.mm. in size. These were immersed in 1% iodine solution for 2 hours to remove mercury pigment. After 1 hour in 5% sodium thiosulphate solution the pieces were washed in distilled water.

The material was then "post-fixed" in 1% osmium tetroxide, buffered with veronal acetate, for 2 hours. Dehydration followed, and was performed according to this schedule:-

3 changes each of 15 mins. in 10% ethanol,

1 period of 15 mins. in 50% ethanol,

3 changes each of 30 mins. in absolute ethanol,

finally, 2 changes each of 20 mins. in 1:2 epoxypropane.

The fixed and dehydrated tissue was next impregnated with "ARALDITE". After leaving overnight at room temperature in polythene "boats"; the impregnated tissue was incubated at 56°C for 30 minutes to evaporate any residual epoxypropane. Embedding in fresh ARALDITE followed, and incubation at 56°C for a further 48 hours.

Sections were cut on a LKB Automatic Ultramicrotome, and collected on ATHENE type 483 E.M. grids. Suitable sections were stained with Reynold's lead citrate (2 mins) and saturated uranyl acetate in 50% ethanol (15 mins).

The grids were examined on an A.E.I. E.M.6 electron microscope and representative micrographs taken.

Electron Microscopy Findings /

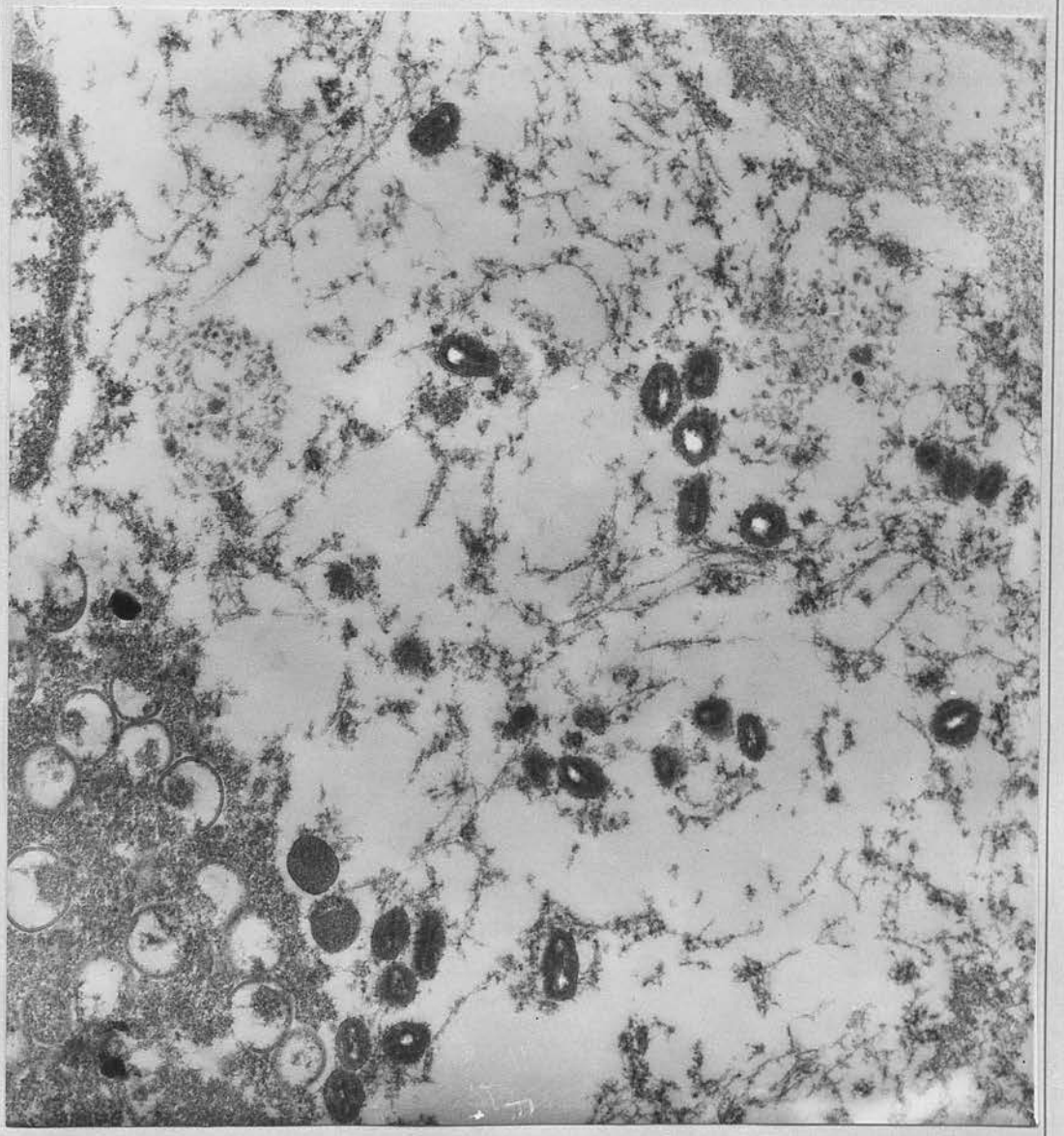

Fig.12. Vaccinia virions within skin. EM. x 32,000.

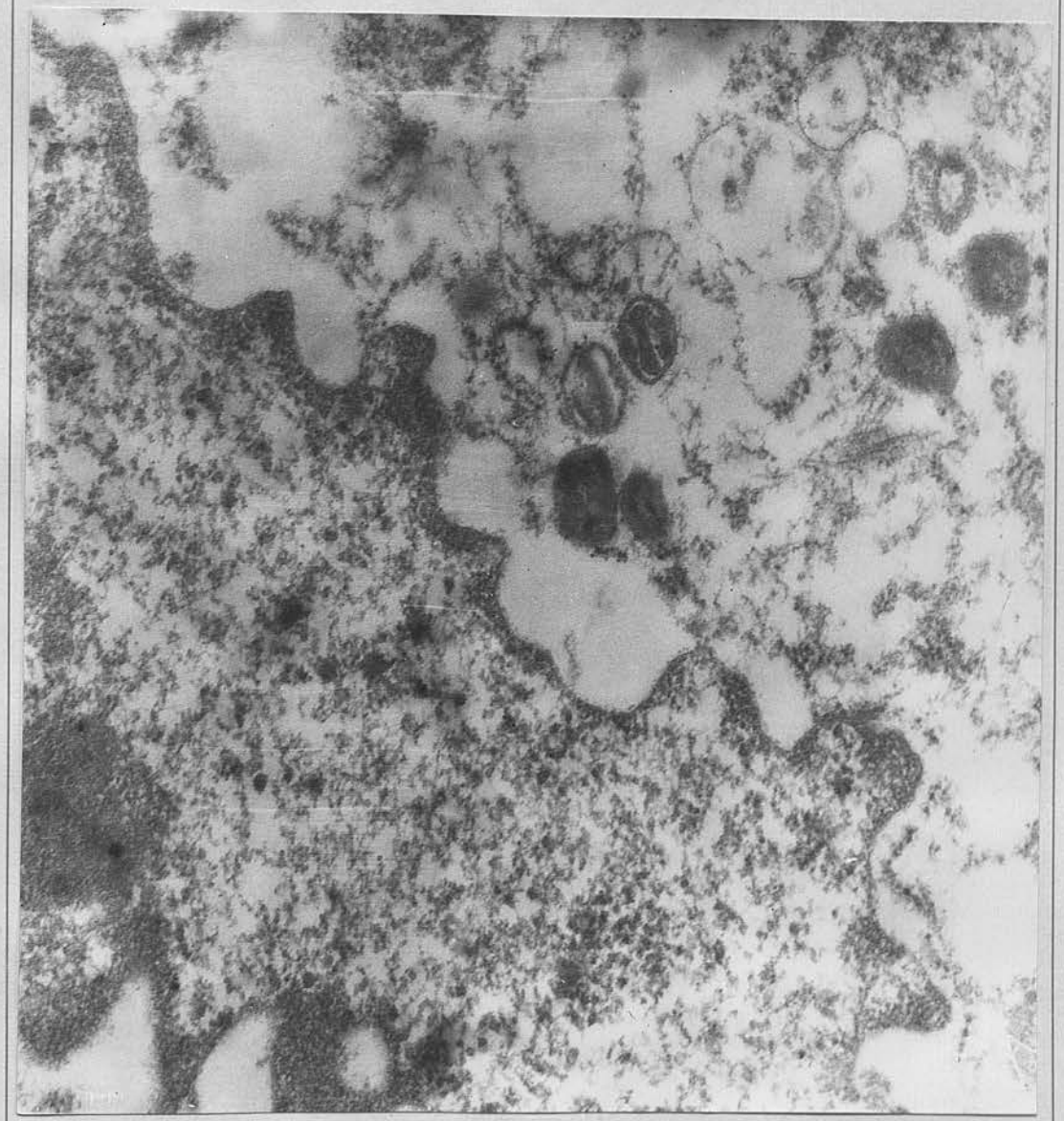

Fig.13. Skin. Higher power showing virion with biconcave nucleoid. EM. x 60,000.

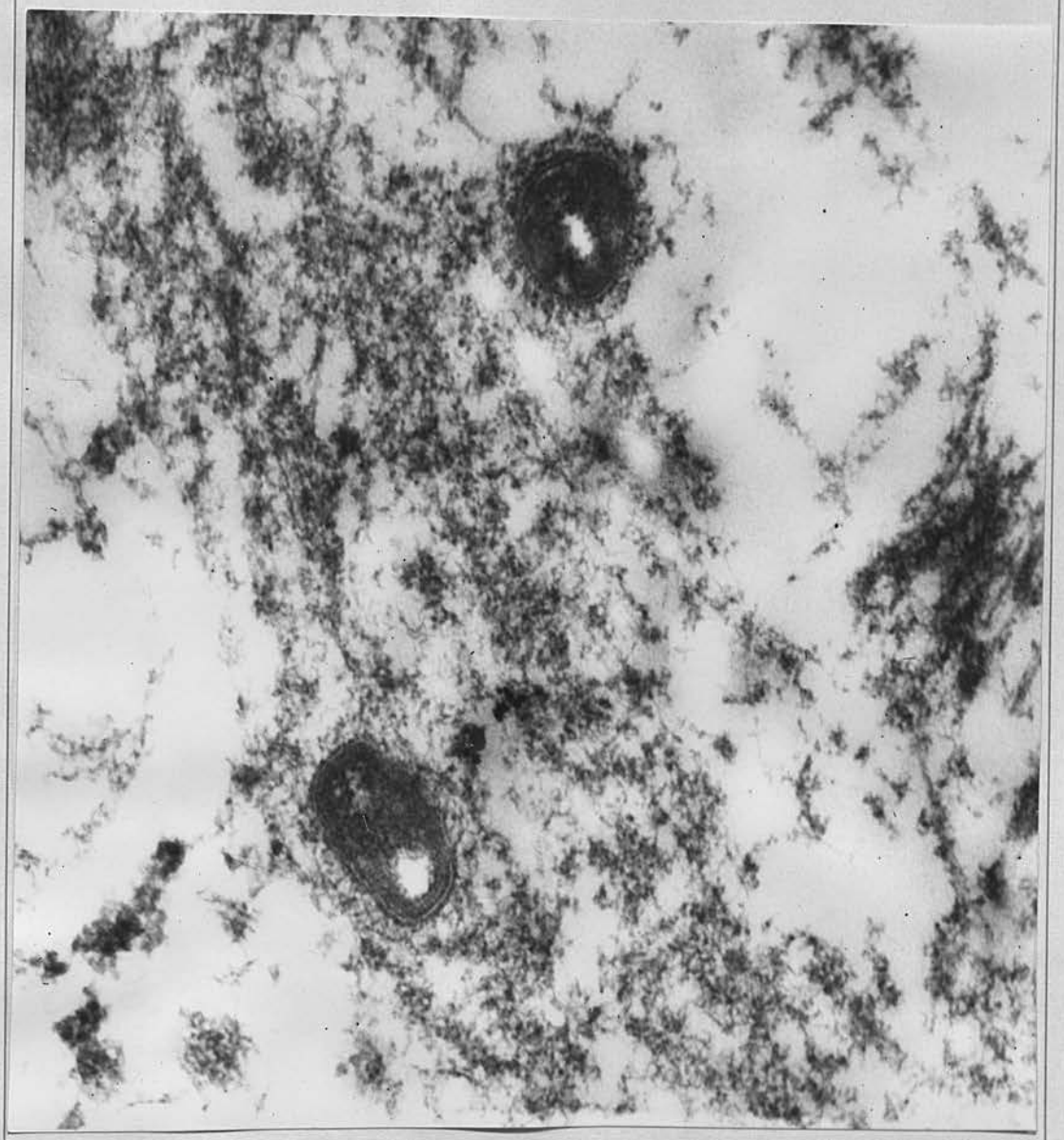

Fig.14. Skin. Two immature virions showing granular viroplasm and double-membrane. EM. x 100,000.

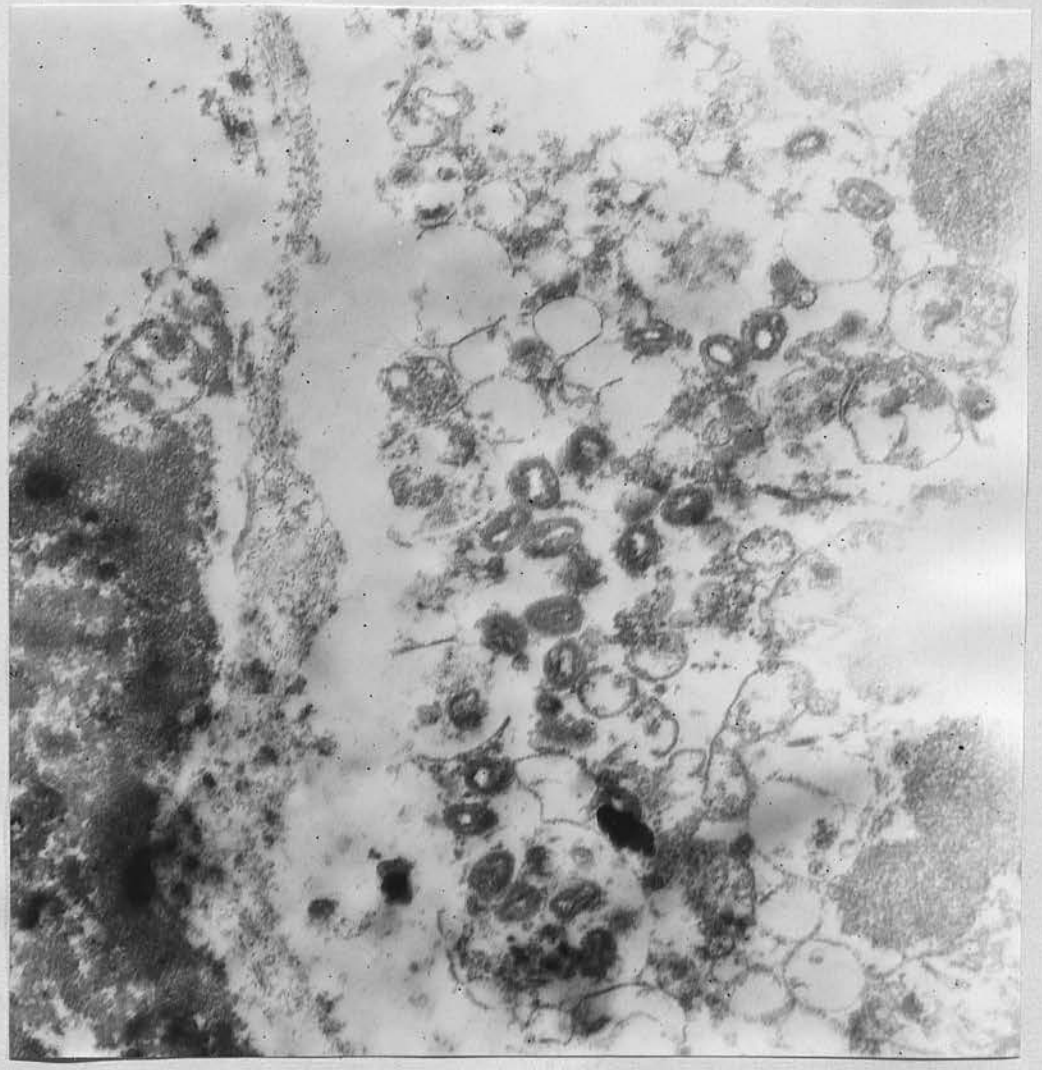

Fig.15. Lung. The field contains numerous virions at various stages of maturation. EM. x 32,000.

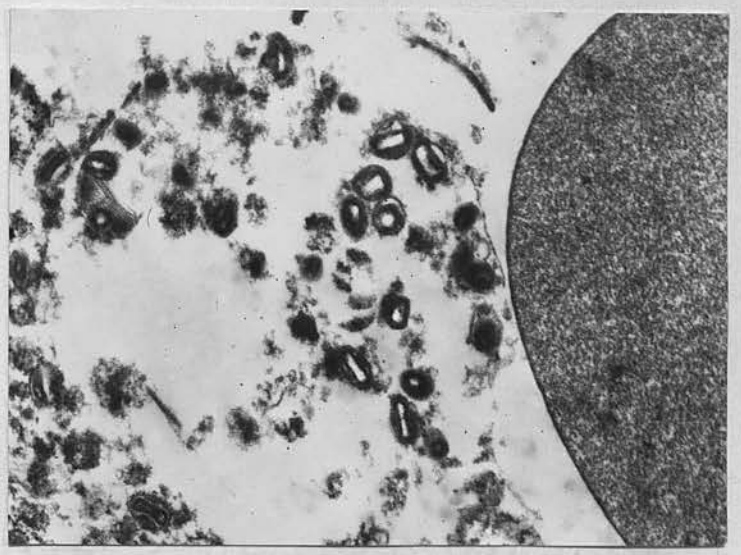

Fig.16. Vaccinia virus within lung tissue. These are largely mature types. The large granular area is part of a red blood cell. EM. x 20,000.

Fig.17. Higher power view of an individual virion showing the characteristic biconcave nucleoid, granular viroplasm, and double membrane. EM. x 100,000.

## Electron Microscopy Findings

In both skin and lung sections large numbers of vaccinia virus were identified. The individual virions vary from 244 to 305 nm in diameter, and various morphological forms are present (figs. 12 - 15 ). The majority are spherical with granular viroplasm enclosed in a double membrane, whilst a smaller number contain a biconcave nucleoid separated from the granular material by a zone of lesser density (figs. 16 and 17).

# DISCUSSION

## Review of the Literature

The last review of the literature was published in 1961 by Erichson and McNamara. They found twelve cases of progressive vaccinia in the English literature, of which three were in adults. Davidson and Hayhoe (1962) cited ten authors who had reported progressive vaccinia accompanying reticuloses and leukaemias. The present survey has revealed forty adult cases in the world literature. They are detailed in the accompanying table and are arranged in relation to the underlying disease.

Ramond, Jacquelin and Borrien (1922) described three patients with malignant lympho-reticular neoplasms in whom vaccination provoked a marked deterioration in their general condition, which in two cases led to death. In these cases, however, the vaccination site, after an initial severe reaction, resolved normally. They ascribed the deleterious effect of vaccination to "a symbiosis between the vaccinia virus and the virus causing the lymphoid neoplasm".

Of further interest is the case reported by Waddington et al. (1964) /

TABLE

Reported cases of progressive vaccinia

| Associated disease | Reference | Age and Sex | Previous treatment | Outcome | Lung involvement |
|---|---|---|---|---|---|
| Acute leukaemia | Davidson and Hayhoe (1962) | 22 M | Steroids; 6-mercapto-purine | Died | ... |
| Chronic lymphatic leukaemia | Beerman and McGuire (1944) | 33 F | Irradiation | ... | ... |
| | | 62 M | Irradiation | ... | ... |
| | Bousser, Christol and Quichaud (1955) | 52 M | Irradiation | Recovered | ... |
| | Olansky, Smith and Hansen-Pruss (1956) | 42 F | Steroids | Died | ... |
| | Blumenthal (1957) | 76 M | ... | ... | ... |
| | Mendansky, Brennar and Kennedy (1960) | 72 M | ... | Died | Miliary lung abscesses; pneumonia; pulmonary oedema |
| | Erichson and McNamara (1961) | 58 M | Irradiation; chemotherapy | Died | Bronchial carcinoma; staphylococcal pneumonia |
| | Vladimirskaya (1961) | 12 cases | ... | ... | ... |
| | Fekety, Malawista and Young (1962) | 66 M | Irradiation; chemotherapy | Recovered | ... |
| | Waddington et al. (1964) | 60 F | None | Recovered | ... |
| | Pedich and Kusch (1966) | 67 M | Chemotherapy | Died | X-ray evidence of "lung necrosis"; no necropsy details |
| | Colon and Lee (1968) | 73 M | ... | Recovered | ... |

TABLE - continued

| Associated disease | Reference | Age and Sex | Previous treatment | Outcome | Lung involvement |
|---|---|---|---|---|---|
| Chronic myeloid leukaemia | Vladimirskaya (1961) | 4 cases | ... | ... | ... |
| Leukaemia (type unspecified) | Kempe (1960) | 3 cases | ... | 2 died | ... |
| Hodgkin's disease | Baum et al. (1966) | 40 F | ... | Recovered | ... |
| | Larsen (1966) | 45 F | Irradiation | Died | ... |
| Giant follicular lymphoma | Daly and Jackson (1962) | 32 M | ... | Recovered | ... |
| Reticular hyperplastic lymphopathy (?) | Pipitone and Russo (1963) | 39 F | Steroids; irradiation | Died | ... |
| Lymphosarcoma | Öberg, Nathorst-Windahl and Wesslén (1958) | 52 F | Irradiation; chemotherapy | Died | Small patches of bronchopneumonia with foci containing "probably aspirated material |
| No associated disease | Conybeare (1938) cited by Lawrence, Cunliffe and Dudgeon (1952) | 31 M | ... | Died | ... |
| | Kempe (1960) | "Elderly" F | ... | Died | ... |
| | O'Connell et al (1964) | 75 F | ... | Recovered | ... |
| | van Rooyen et al (1967) | 55 F | ... | Died | Several small infarcts with multifocal alveolar haemorrhages |

(1964) of progressive vaccinia in a boy of thirteen with acute leukaemia, who had received large doses of steroids, methotrexate and 6-mercaptopurine prior to vaccination.

Histology and electron-microscope findings

The majority of reports of progressive vaccinia have been clinically orientated and contain little detailed description of post-mortem findings. A comprehensive pathological description is included in a case of progressive vaccinia in a child of 6 mth reported by Hall, Cunliffe and Dudgeon (1953) and their findings parallel closely those observed in the present case. In the cutaneous lesions, for example, they found a complete absence of inflammatory cells. Whilst small numbers of lymphocytes were found in the present case, it appears that absence of polymorphonuclear leucocytes, and a diminished or absent lymphocytic response, are constant features of progressive vaccinia.

The inclusion bodies of vaccinia have been fully described by Kamahora et al. (1958) and Kato et al. (1959). They demonstrated that the Guarnieri body of vaccinia is an intra-cytoplasmic "B"-type inclusion; that is, it is irregular in shape, haematoxylinophilic, and stains reddish-purple with Giemsa stain. The finding of round circumscribed eosinophilic inclusions ("A" type) is rare in vaccinia, and then occurs only if the strain is of cowpox, as opposed to variola, origin.

Although inclusion bodies were not found in this case or in the case of Hall et al., their absence is not a constant feature of /

of progressive vaccinia, as they are stated to have been found in the skin of a child reported by Bigler and Slotkowski (1951).

A further point of similarity between the present case and that of Hall et al. is the finding of foci of necrosis within the liver. These foci, which are unusual in that they lack an associated inflammatory cell response, have also been described in human vaccinial infection by Dible and Gleave (1934), and in experimental vaccinia in rabbits by Lillie and Armstrong (1930). Although foci of necrosis are found in the liver in salmonellosis, they are usually associated with macrophage granulomata, which were not present in this case.

The histology of the spleen is difficult to evaluate and appears to have been modified by a prolonged stress reaction and steroid therapy. Some features are suggestive of an endotoxaemia, but the absence of polymorphonuclear leucocytes in the spleen, and of fibrin in the renal glomerular capillaries, do not support this diagnosis. Thus, the histological findings indicate that the terminal Salmonella typhimurium bacteriaemia was of limited pathological significance.

The most interesting features of the present case are the widespread and severe lung changes. Whilst lung lesions were present in five of the adult cases of progressive vaccinia, a detailed microscopic description of these lesions is not included in any of the reports. In only one case, that reported by Öberg, Nathorst-Windahl and Wesslén (1958), was virus isolated from the lung and this was from "probably aspirated material". The presence of extensive serofibrinous oedema and a scanty

macrophagic /

macrophagic response are appearances similar to those found in the human infection by Hall et al., and in experimental vaccinia in rabbits by Lillie and Armstrong. Hyperplasia of the bronchial epithelium has not previously been observed in human vaccinial infection, but has been seen in the rabbit (Montasir, Rabin and Phillips, 1966).

The histological appearances were suggestive of a vaccinial pneumonia, and electron microscopy confirmed the presence of virus particles. Lung sections revealed large numbers of virions, and a variety of morphological forms were observed similar to those found in experimental vaccinial pneumonia in mice by Montasir et al. The various morphological forms are related to the sequence of maturation as described in the chick chorio-allantois by Gaylord and Melnick (1953) and Morgan et al. (1954). Large numbers of virus particles were also identified in the sections of skin. This is the first case of progressive vaccinia in which vaccinia virus has been demonstrated in post-mortem material by means of electron microscopy.

## Immunological aspects

Defective antibody formation is a well-established feature of both the reticuloses (Evans, 1948; Silver et al., 1958; Barr and Fairley, 1961) and leukaemias (Howell, 1920; Shaw, 1960). The association of progressive vaccinia with such disorders, and its increased frequency in children with agammaglobulinaemia (Kempe, 1960) led a number of authors to conclude that a failure in formation of neutralising antibodies was a sine qua non for the development of this complication. For example, a typical definition of progressive vaccinia describes it as "An extremely rare complication of smallpox vaccination ... characterised by a complete inability to make specific antibodies against the vaccinia virus" (Kempe and Benenson, 1955).

Four cases have been reported, however, where progressive vaccinia has occurred despite the presence of adequate levels of circulating neutralising antibodies, three in adults (Daly and Jackson, 1962; Davidson and Hayhoe, 1962; O'Connell et al., 1964), and one in a child (Hansson, Johannson and Vahlquist, 1966). It therefore appears that a normal gamma-globulin level is no guarantee against the occurrence of progressive vaccinia. In addition, patients with agammaglobulinaemia often have a normal primary response to vaccination and develop an immune response on repeat vaccination despite the absence of detectable serum antibodies (Apt, 1953).

Further evidence against a major role for antibody in primary vaccinial infection is provided by animal experiments. Irradiated guinea-pigs /

guinea-pigs in which no neutralising antibody could be demonstrated recovered from vaccinia virus infection as rapidly as did normal animals; the irradiated guinea-pigs did, however, develop delayed hypersensitivity to vaccinia during recovery (Friedman and Baron, 1961). Similarly, chick embryos may recover from a variety of viral infections, including vaccinia, although they are unable to produce antibodies (Baron and Isaacs, 1961).

These findings have prompted suggestions that delayed hypersensitivity may be more important than antibody in recovery from vaccinial infection (Dienes and Naterman, 1936; Kempe). Clinical support for this view is provided by the cases of O'Connell et al. and Hansson et al., where, although the patients had an adequate titre of neutralising antibody, scratch tests with inactivated virus failed to produce a delayed skin reaction. O'Connell et al. postulated a causal relationship between failure of the cellular immune response and the development of progressive vaccinia. Kempe reported a case in which the injection into the edge of the necrotic skin lesion of leucocytes and lymph-gland material from immune donors produced a dramatic improvement, and O'Connell et al. attributed the benefit of blood transfusion in their patient to the transfer of immune leucocytes. The ability of immunologically competent cells to alter the course of the disease confirms the importance of cellular immunity in recovery from vaccinial infection.

The results of experimental work on cellular responses have been contradictory. Turk, Allison and Oxman (1962) produced delayed /

delayed hypersensitivity in guinea-pigs in the absence of demonstrable antibody, and showed that there was little difference in the quantity of virus that multiplied in these animals as compared with normal animals. Similarly, Friedman et al. (1962) showed that guinea-pigs in which antibody production and delayed hypersensitivity were blocked recovered as well as normal animals did. These authors concluded that cellular immunity is not a major factor in recovery from primary vaccinial infection. Flick and Pincus (1963), Pincus and Flick (1963) and Pincus, Flick and Ingalls (1963) differ sharply from Friedman et al., for they reported a series of experiments that clearly demonstrated the importance of cellular immunity. They found that the ability of various inbred strains of rabbits to develop rapid and severe reactions to vaccinia was directly proportional to their ability to mount delayed hypersensitivity to other antigens. They also demonstrated that techniques that inhibited the expression of cellular immunity, e.g., inoculation of "antimononuclear-cell" serum locally or the induction of neonatal immunological unresponsiveness, inhibited the primary vaccinia lesion. Hirsch et al. (1968) administered rabbit anti-mouse-thymocyte serum to mice to suppress host cell-mediated responsiveness to intravenously administered vaccinia virus, and found increased morbidity and mortality in the treated animals. There was no parallel effect on either antibody or interferon production in response to vaccinia virus in these mice.

On balance theclinical and experimental evidence suggests that cellular /

cellular immunity is an important factor determining recovery from primary vaccinial infection. The depression of delayed hypersensitivity observed in patients with reticuloses (Rostenberg and Bluefarb, 1954; Epstein, 1958; Fairley and Matthias, 1960; Aisenberg, 1966) therefore offers one explanation for the consistent association between these diseases and the development of progressive vaccinia. This depression of delayed hypersensitivity is possibly due to failure of the neoplastic lymphoid cell to function as an effector of cellular immunity. Fairley (1969) suggests, however, that in cases of Hodgkin's disease most lymphocytes may be committed to react against the neoplastic tissue and are therefore unable to react against other antigens, and that this explains the gross impairment of cellular immunity in these patients.

Cellular immunity, however, is not the only factor determining recovery from viral infections, and there is increasing evidence that non-immune mechanisms play a fundamental part. The most important of these is interferon production.

The protective effect of interferon against vaccinial infection has been demonstrated both by intradermal administration (Isaacs and Westwood, 1959; Andrews, 1961) and by stimulation of endogenous systemic interferon production (Petralli, Merigan and Wilbur, 1965). That interferon production is defective in progressive vaccinia is indicated by a recent case in which the interferon-producing capacity of leucocytes was found to be markedly depressed (van Rooyen et al., 1967). Whilst such findings suggest that administration of interferon to these patients would be beneficial, use of exogenous interferon in a child with progressive vaccinia failed to influence the /

the progress of the disease (Conolly, Dick and Field, 1962). However, only a low final serum titre of interferon was achieved in this case.

Defective production of interferon might be the most important factor in the development of progressive vaccinia. How then is defective production linked with the reticuloses and myeloproliferative disorders?

Interferon production is initiated by the action on cellular metabolism of an "inducer". This inducer has been identified as the nucleoprotein component of the virus (Burke et al., 1968).

Though all cells are capable of interferon synthesis, the finding that the reticulo-endothelial system is the main source of systemically induced interferon is of considerable interest (Kono and Ho, 1965). It is thought that macrophages trap and concentrate the inducer and make it available to the interferon producing cells (Ho, Postik and Ke, 1968), a mechanism analogous to the trapping of antigens in antibody-forming organs to facilitate the production of antibody. Just as depression of phagocytic activity leads to a reduction of antibody production (Stuart and Davidson, 1964) diminished phagocytosis by macrophages results in deficient interferon production (Kono and Ho).

The status of phagocytic activity in the reticuloses is in some doubt. Groch, Perillie and Finch (1965) found depressed phagocytic activity in patients with leukaemia, lymphoma and multiple myeloma, whilst others have demonstrated a hyper-phagocytic state in similar cases (Salky et al., 1967; Sheagren, Block and Wolff, 1967). These discrepancies may result from difficulty in assessing /

assessing the stage of the disease, for in mouse leukaemia enhanced phagocytosis is seen in the early stages of the disease, but as the involvement progresses phagocytic activity diminishes (Old et al., 1960).

Deficient production of systemic interferon, even in the presence of normal or increased phagocytosis, might ultimately be due to a failure of neoplastic cells to respond to the inducer. This hypothesis is supported by the finding that leucocytes from patients with leukaemia and lymphoma exhibit reduced interferon production as compared with normal leucocytes after in-vitro stimulation by virus (Hayashi, Sharples and Lo Grippo, 1966; Lee, Ozere and van Rooyen, 1966). With the recent development of precise methods for interferon quantitation (Finter, 1966) a study of systemic interferon production in patients with reticuloses and leukaemias would be of considerable interest.

Thus far, only the impairment of immune responses brought about by the underlying disease has been discussed. A further important consideration is the effect on immune reactivity of corticosteroids, antimetabolite chemotherapy and irradiation, for at least ten of the reported cases had received such treatment.

The enhancing effect of corticosteroids on experimental vaccinial infection has been clearly demonstrated (Rose et al., 1952; Bugbee, Like and Stewart, 1960). Corticosteroids have been shown to depress antibody formation (Bjørneboe, Fischel and Stoerk, 1951) and to cause lymphopenia and involution of lymphoid tissue (Dougherty, 1953). Of more importance is the finding that cortisone administration markedly depressed the interferon response in /

in experimental animals (Burke, 1966; Rytel and Kilbourne, 1966; Lavelle and Starr, 1968). Whilst this effect may be due to direct suppression of interferon production, the striking depression of phagocytic activity brought about by corticosteroids (Heller, 1955; Nicol, Snell and Bilbey, 1956; Nicol and Bilbey, 1958) indicates that a failure to trap and concentrate viral inducer might be the predominant factor involved.

Antimetabolites have similarly been shown to impair immune responses. Depression of antibody production (Condie, Mennis and Miller, 1961) inhibition of lymphocyte response (Page, Condie and Good, 1962; Page, 1964) and reduced phagocytic activity (Megirian, Walton and Lang, 1959; Megirian, 1965), have been demonstrated by the use of a variety of antimetabolic drugs.

Although irradiation leads to diminished antibody production and lymphopenia (Wilson and Miles, 1946), its effects on interferon production are variable, and at therapeutic levels probably has little influence. Hellman, Martin and Wopschall (1968) found no significant alteration in interferon levels in irradiated animals.

Thus, the state of altered immunity predisposing to the development of progressive vaccinia includes both a deficient cellular response and depressed interferon production. These changes are largely attributable to the underlying lymphoreticular or myeloproliferative disease, but the additional impairment resulting from the administration of corticosteroids, anti-metabolite drugs, and possibly from irradiation constitutes an important factor in the pathogenesis of this rare complication.

The potentially fatal complications of vaccination could be avoided /

avoided by using an inactivated vaccine. Unfortunately the development of an effective heat-killed vaccine has met with only partial success (Madely, 1968). Until such a vaccine is available, patients suffering from a reticulosis or leukaemia, under treatment with immunosuppressive drugs, or undergoing irradiation, should be warned of the dangers of anti-smallpox vaccination.

## REFERENCES


Aisenberg, A.C. 1966. Immunologic status of Hodgkin's disease. Cancer, Philad., 19, 384.

Andrews, R.D. 1961. Specificity of interferon. Br.Med.J., 1, 1728.

Apt, L. 1953. Agammaglobulinaemia. Yearbook of Pediatrics. Chicago, p. 67.

Baron, S., and Isaacs, A. 1961. Mechanism of recovery from viral infection in the chick embryo. Nature, Lond., 191, 97.

Barr, Mollie, and Fairley, G.H. 1961. Circulating antibody in reticuloses. Lancet, 1, 1305.

Baum, P., and Meyer zum Buschenfelde, 1966. Vaccinia gangraenosa et generalisata bei Lymphogranulomatose. Med.Welt, 16, 878.

Beerman, H., and McGuire, J.A. 1944. Chronic lymphatic leukaemia complicated by vaccinia. Archs Derm.Syph., Chicago, 49, 288.

Bigler, J.A., and Slotkowski, E.L. 1951. Smallpox vaccination with prolonged vaccinia. Pediatrics, 7, 24.

Bjørneboe, M., Fischel, E.E., and Stoerk, H.C. 1951. Effect of cortisone and adreno-corticotrophic hormone on concentration of circulating antibody. J.Exp.Med., 93, 37.

Blumenthal, F., et al. 1957. Gangrenous vaccination. Archs Derm. Syph., Chicago, 76, 124.

Bousser, J., Christol, D., and Quichaud, J. 1955. Accidents locaux et généraux de la vaccination jennérienne chez les leucosiques; à propos d'une observation récente. Presse méd., 63, 1797.

Bugbee, L.M., Like, A.A., and Stewart, R.B. 1960. The effect of cortisone on intradermally induced vaccinia infection in rabbits. J.Infect.Dis., 106, 166.

Burke, D.C. 1966. The production of interferons by viruses: Mechanisms. In Interferons, ed. by N.B. Finter, Amsterdam, p.79.

Burke, D.C., Skehel, J.J., Hay, A.J., and Walters, S. 1968. Cellular events preceding interferon formation. In Interferon, Ciba Fdn Symp., p.4.

Colon, V.F., and Lee, L.R. 1968. Vaccinia necrosum as a clue to lymphatic lymphoma. Geriatrics, 23, 81.


Condie, R.M., /

Condie, R.M., Mennis, W.I., and Miller, Carolyn. 1961. Effect of 6-mercaptopurine (6-MP) on antibody production. Fedn Proc., 20, 26.

Connolly, J.H., Dick, G.W.A., and Field, G.M.B. 1962. A fatal case of progressive vaccinia. Br.Med.J., 1, 1315.

Daly, J.J., and Jackson, E. 1962. Vaccinia gangrenosa treated with N-methylisatin-semicarbazone. Br.Med.J., 2, 1300.

Davidson, E., and Hayhoe, F.G.J. 1962. Prolonged generalized vaccinia complicating acute leukaemia. Br.Med.J., 2, 1298.

Dible, J.H., and Gleave, H.H. 1934. Histological and experimental observations upon generalised vaccinia in man. J.Path.Bact., 38, 29.

Dienes, L., and Naterman, H.L. 1936. Immunological response to vaccinia in guinea-pigs. J.Infect.Dis., 60, 279.

Dougherty, T.F. 1953. Some observations on mechanisms of cortico-steroid action on inflammation and immunologic processes. Ann. N.Y. Acad.Sci., 56, 748.

Epstein, W.L. 1958. Induction of allergic contact dermatitis in patients with the lymphoma-leukaemia complex. J.Invest.Derm., 30, 39.

Erichson, R.B., and McNamara, M.J. 1961. Vaccinia gangrenosa. Report of a case and review of the literature. Ann.Intern. Med., 55, 491.

Evans, R.W. 1948. Antibody response in cases of radiation lymphopenia and in reticuloses. J.Path.Bact., 60, 123.

Fairley, G.H. 1969. Immunity to malignant disease in man. Br.Med.J., 2, 467.

Fairley, G.H., and Matthias, J.Q. 1960. Cortisone and skin sensitivity to tuberculin in reticuloses. Br.Med.J., 2, 433.

Fekety, F.R., Malawista, S.E., and Young, D.L. 1962. Vaccinia gangrenosa in chronic lymphatic leukemia; report of a recovery following the use of vaccinia immune globulin. Archs Intern. Med., 109, 205.

Finter, N.B. 1966. Interferon assays and standards. In Interferons, Amsterdam, p.87.

Flick, J.A., /

Flick, J.A., and Pincus, W.B. 1963. Inhibition of the lesions of primary vaccinia and of delayed hypersensitivity through immune tolerance in rabbits. J.Exp.Med., 117, 633.

Friedman, R.M., and Baron, S. 1961. The role of antibody in recovery from infection with vaccinia virus. J.Immune., 87, 379.

Friedman, R.M., Baron, S., Buckler, C.E., and Steinmuller, R.L. 1962. The role of antibody, delayed hypersensitivity and interferon production in recovery of guinea-pigs from primary infection with vaccinia virus. J.Exp.Med., 117, 347.

Gaylord, W.H., and Melnick, J.L. 1953. Intracellular forms of pox viruses as shown by the electron microscope (vaccinia, ectromelia, molluscum contagiosum). J.Exp.Med., 98, 157.

Groch, C.S., Perillie, P.E., and Finch, S.C. 1965. Reticulo-endothelial phagocytic function in patients with leukemia, lymphoma and multiple myeloma. Blood, 26, 489.

Hall, G.F.M., Cunliffe, A.C., and Dudgeon, J.A. 1953. Prolonged generalised vaccinia. J.Path.Bact., 66, 25.

Hansson, O., Johansson, S.G.O., and Vahlquist, B. 1966. Vaccinia gangraenosa with normal humoral antibodies. A case possibly due to deficient cellular immunity treated with N-methylisatin beta-semicarbazone (compound 33T57, Marboran). Acta paediat. scand., 55, 264.

Hayashi, H., Sharples, N.S., and LoGrippo, G.A. 1966. Comparative study between immunoglobulin status and interferon response in health and disease. J.Reticuloendothel.Soc., 3, 1.

Heller, J.H. 1955. Cortisone and phagocytosis. Endocrinology, 56, 80.

Hellman, A., Martin, D.H., and Wopschall, L.J. 1968. The influence of X-irradiation on survival and interferon levels in viral infected mice. Proc.Soc.Exp.Biol.Med., 128, 455.

Hirsch, M.S., Nahmias, A.J., Murphy, A., and Kramer, J.H. 1968. Cellular immunity in vaccinial infection of mice. Anti-lymphocytic serum and secondary responsiveness. J.Exp.Med., 128, 121.

Ho, M., Postic, B., and Ke, Y.H. 1968. The systemic induction of interferon. In Interferon, Ciba Fdn Symp., p.19.

Howell, Katherine M. 1920. The failure of antibody formation in leukaemia. Archs Intern. Med., 26, 706.

Isaacs, A., and Westwood, Marguerite A. 1959. Inhibition by interferon of the growth of vaccinia virus in rabbit skin. Lancet, 2, 324.

Kamahora, J., /

Kamahora, J., Sato, Y., Kato, S., and Hagiwara, K. 1958. Inclusion bodies of the vaccinia virus. Proc.Soc.Exp. Biol.Med., 97, 43.

Kato, S., Takahashi, M., Kameyama, S., and Kamahora, J. 1959. A study on the morphological and cytoimmunological relationship between the inclusions of variola, cowpox, rabbitpox, vaccinia, and a consideration of the term "Guarnieri Body". Biken's J., 2, 353.

Kempe, C.H. 1960. Studies on smallpox and complications of smallpox vaccination. Paediatrics, 26, 176.

Kempe, C.H., and Benenson, A.S. 1955. Symposium on unusual infections of childhood: smallpox and vaccinia. Pediat. Clins N.Amer., 2, 19.

Kono, Y., and Ho, M. 1965. The role of the reticuloendothelial system in interferon formation in the rabbit. Virology, 25, 162.

Larsen, A.A. 1966. A severe complication of smallpox vaccination. Canad.Med.Assoc.J., 94, 1316.

Laurance, B., Cunliffe, A.C., and Dudgeon, J.A. 1952. Vaccinia gangrenosa. The report of a case of prolonged generalized vaccinia. Archs Dis. Childh., 27, 482.

Lavelle, G.C., and Starr, T.J. 1968. Effects of cortisone on interferon response of germ-free mice to mouse hepatitis virus. J. Reticuloendothel.Soc., 5, 436.

Lee, S.H.S., Ozere, R.L., and van Rooyen, C.E. 1966. Interferon production by human leucocytes in vitro. Reduced levels in lymphatic leukemia. Proc.Soc.Exp.Biol.Med., 122, 32.

Lillie, R.D., and Armstrong, C. 1930. The pathology of generalised vaccinia in rabbits. Natn.Inst.Hlth.Bull., no.156.

Madely, C.R. 1968. The immunogenicity of heat-inactivated vaccinia virus in rabbits. J.Hyg., Camb., 66, 89.

Mendansky, R.S., Brennan, Betsy B., and Kennedy, R.P. 1960. Severe vaccination reaction in a leukemic patient. Archs Derm.Syph., Chicago, 81, 601.

Megirian, R. 1965. Effect of chlorambucil on the blood clearances of injected particulate matter. J.Reticuloendothel.Soc., 2, 238.

Megirian, R., Walton, Mae S., and Lang, E.P. 1959. The effect of eight anticancer substances on the phagocytic properties of the reticuloendothelial system. J.Pharmac.Exp.Ther., 127, 81.

Montasir, M., /

Montasir, M., Rabin, E.R., and Phillips, C.A. 1966. Vaccinia pneumonia in mice. A light and electron microscopic and viral assay study. Amer.J.Path., 48, 877.

Morgan, C., Ellison, S., Rose, H., and Moore, D. 1954. Structure and development of viruses observed in electron microscope: vaccinia and fowl pox viruses. J.Exp.Med., 100, 301.

Nicol, T., and Bilbey, D.L.J. 1958. Substances depressing the phagocytic activity of the reticulo-endothelial system. Nature, Lond., 182, 606.

Nicol, T., Snell, R.S. and Bilbey, D.L.J. 1956. Effect of cortisone on defence mechanisms of the body. Br.Med.J., 2, 800.

Oberg, G., Nathorst-Windahl, G., and Wesslén, T. 1958. Vaccinia gangraenosa: en sallsynt komplikation efter variolavaccination. Nord.Med., 60, 1045.

O'Connell, C.J., Karzon, D.T., Barron, A.L., Plant, M.E. and Ali, V.M. 1964. Progressive vaccinia with normal antibodies. A case possibly due to deficient cellular immunity. Ann. Intern.Med., 60, 282.

Olansky, S., Smith, J.G., Jr, and Hansen-Pruss, O.C.E. 1956. Fatal vaccinia associated with cortisone therapy. J.Amer. Med.Assoc., 162, 887.

Old, L.J., Clarke, D.A., Benacerraf, B., and Goldsmith, Marilyn. 1960. The reticulo-endothelial system and the neoplastic process. Ann.N.Y.Acad.Sci., 88, 264.

Page, A.R. 1964. Inhibition of the lymphocyte response to inflammation with antimetabolites. Amer.J.Path., 45, 1029.

Page, A.R., Condie, R.M., and Good, R.A. 1962. Effect of 6-mercaptopurine on inflammation. Amer.J.Path., 40, 519.

Pedich, W., and Kusch, F. 1966. Vaccinia gangraenosa w przypadku przwlekteklej bialatczki limfacznej. Przegl.Lek., 22, 319 (in Polish).

Petralli, J.K., Merigan, T.C., and Wilbur, J.R. 1965. Action of endogenous interferon against vaccinia infection in children. Lancet, 2, 401.

Pincus, W.B., and Flick, J.A. 1963. Inhibition of the primary vaccinial lesion and of delayed hypersensitivity by an antimononuclear cell serum. J.Infect.Dis., 113, 15.

Pincus, W.B., /

Pincus, W.B., Flick, J.A., and Ingalls, T.H. 1963. The response of inbred rabbits to vaccinia virus infection. J.Immun., 91, 58.

Pipitone, V., and Russo, R. 1963. Su di un caso di vaccino gangrenoso. Boll. Ist. sier. milan., 42, 233. (in Italian).

Ramond, F., Jacquelin, D., and Borrien. 1922. Accidents graves de la vaccination jennérienne au cours de certains lymphadénies. Bull.Mém.Soc.méd.Hop.Paris, 66, 65.

Rose, H.M., Holden, M., Blunt, J.W., and Ragan, C.A. 1952. The effect of cortisone on infection with vaccinia virus in rabbits. Bact.Proc., 53, 81.

Rostenberg, A., and Bluefarb, S.M. 1954. Cutaneous reactions in lymphoblastomas. Archs Derm.Syph., Chicago, 69, 195.

Rytel, M.W., and Kilbourne, E.D. 1966. The influence of cortisone on experimental viral infection. 8. Suppression by cortisone of interferon formation in mice injected with Newcastle disease virus. J.Exp.Med., 123, 767.

Salky, N.K., DiLuzio, N.R., Levin, A.G., and Goldsmith, H.S. 1967. Phagocytic activity of the reticuloendothelial system in neoplastic disease. J.Lab.Clin.Med., 70, 393.

Shaw, R.K., Szwed, C., Boggs, D.R., Fahey, J.L., Frei, E., III, Morrison, Eleanor, and Utz, J.P. 1960. Infection and immunity in chronic lymphocytic leukemia. Archs Intern.Med., 106, 467.

Sheagren, J.H., Block, J.B., and Wolff, S.M. 1967. Reticuloendothelial system phagocytic function in patients with Hodgkin's disease. J.Clin.Invest., 46, 855.

Silver, R.T., Utz, J.P., Frei, E., III, and McCullough, N.B. 1958. Fever, infection and host resistance in acute leukemia. Amer. J.Med., 24, 25.

Stuart, A.E., and Davidson, A. Edna. 1964. Effect of simple lipids on antibody formation after injection of foreign red cells. J.Path.Bact., 87, 305.

Topley, W.W.C., and Wilson, G.S. 1946. Principles of bacteriology and immunity, 3rd ed., revised by G.S. Wilson and A.A. Miles, London, p. 1104.

Turk, J.L., Allison, A.C., and Oxman, M.N. 1962. Delayed hypersensitivity in relation to vaccination and multiplication of vaccinia virus in the guinea-pig. Lancet, 1, 405.

van Rooyen, C.E., Casey, Joan, Lee, S.H.S., Faulkner, Ruth, and Dincsoy, H.P. 1967. Vaccinia gangrenosa and 1-methylisatin 3-thiosemicarbazone (methisazone). Canad.Med.Assoc.J., 97, 160.

Vladimirskaya, E.B. /

Vladimirskaya, E.B. 1961. Complications in leukaemic patients vaccinated against smallpox. Sov.Med., 25, 118 (in Russian).

Waddington, E., Bray, P.T., Evans, A.D., and Richards, I.D.G. 1964. Cutaneous complications of mass vaccination against smallpox in South Wales, 1962. Trans. A. Rep. St John's Hosp. Derm. Soc., Lond., 50, 22.